UNITED STATES DEPARTMENT OF AGRICULTURE

FOREST SERVICE

4110
FS-SO-1401-5.18

FINAL REPORT

EFFECTS OF CERATOCYSTIS MINOR (HEDGC.) HUNT, THE BLUE STAIN FUNGUS CARRIED BY DENDROCTONUS FRONTALIS ZIMM. ON BEETLE-RESISTANT AND -SUSCEPTIBLE PINE SPECIES

Robert C. Hare
Plant Physiologist

Institute of Forest Genetics
Gulfport, Mississippi

Southern Forest Experiment Station

6200-7 (6/65)

U.S. GOVERNMENT PRINTING OFFICE : 1965 O—777-887

4110
FS-SO-1401-5.18

FINAL REPORT

EFFECTS OF CERATOCYSTIS MINOR (HEDGC.) HUNT, THE BLUE STAIN FUNGUS CARRIED BY DENDROCTONUS FRONTALIS ZIMM. ON BEETLE-RESISTANT AND -SUSCEPTIBLE PINE SPECIES

Prepared by:

Robert C. Hare — 10/30/69 (Date)
Robert C. Hare
Plant Physiologist

Approved:

Dan Schmitt — 10-30-69 (Date)
Dan Schmitt, Project Leader
Institute of Forest Genetics

FINAL REPORT

EFFECTS OF CERATOCYSTIS MINOR (HEDGC.) HUNT, THE BLUE STAIN FUNGUS CARRIED BY DENDROCTONUS FRONTALIS ZIMM. ON BEETLE-RESISTANT AND -SUSCEPTIBLE PINE SPECIES

Because my research project on the physiology of insect resistance was terminated in a short time, work on this study plan was not completed. This report presents the limited data obtained.

OBJECTIVES

As described in the study plan, dated 2-19-65, the main objective was to study the susceptibility of pines to pure cultures of C. minor. Shortleaf and loblolly pines, highly susceptible to the southern pine beetle, were compared with slash pine, moderately beetle-resistant, to see if there is any correlation between susceptibility to the beetle and to the fungus. If so, killing of susceptible trees by beetles might be indirect, from the fungal infection introduced by the beetle. If this were true resistance to blue-stain would automatically confer resistance to beetle-kill.

Secondary objectives were to compare the effects of wood extracts from beetle-resistant and -susceptible species on spore germination and mycelial growth of C. minor in vitro, and to measure the effects of C. minor infection on oleoresin flow and viscosity in the three species. No work was done on the last objective.

METHODS AND RESULTS

Isolation and culture of C. minor

As described in the study plan, cultures of C. minor were obtained by flaming excised bits of firm blue-stained wood in alcohol and culturing on sterile malt agar. Uncontaminated subcultures were made by transferring from isolated colonies of C. minor. Internal contamination (mainly Trichoderma) was a major problem, and much of the blue-stained wood was apparently free of C. minor at the time of sampling. Other methods of surface sterilization tested (soaking in H_2O_2 0.35 to 35 percent or NaOCl 5.25 percent) were either ineffective or gave complete sterilization. Some colonies of C. minor were obtained by allowing southern pine beetles to walk over malt agar plates.

C. minor grew slowly on Difco malt agar. When enriched with pine wood extract growth was much better. Extracts were made by shaving pencil-sized twigs of southern pines in a pencil sharpener, boiling 100 g. of the shavings in 500 ml. water 15 minutes, and filtering. The filtrate was autoclaved with 1 percent malt agar.

In a test of species effects 5 mm. discs were cut with a sterile cork borer from C. minor cultures and placed in the center of petri dishes containing wood-extract malt agar. After 1 week the diameter of the colonies was measured (table 1). All extracts except shortleaf promoted growth over that on malt agar. Differences were significant at the 0.01 level. White conidiophores developed in 1 day on the slash and loblolly pine extracts, somewhat slower on longleaf, and much slower on shortleaf.

Table 1.--Effect of wood extracts on 7-day growth of C. minor on malt extract agar. Mean of 4 plates. Means connected by bar not significantly different at the 0.05 level

Source of extract species	Size of colony mm.	Appearance of colony
Control	13.8	Gray, very sparse
Shortleaf	14.2	Gray-white, sparse
Longleaf	20.0	White, thicker
Slash	21.2	Pure white, fluffy
Loblolly	26.0	Pure white, fluffy

The malt agar control showed much less of the white sporulating mycelium. According to Duncan's Multiple Range Test colony growth on loblolly extract was significantly greater, and that on shortleaf and the control was less, than that on slash and longleaf, at the 0.05 level.

Although the species used for the extract affected growth of C. minor there was no correlation between susceptibility to the southern pine beetle and promotion of C. minor growth by the wood extracts. Thus the shortleaf and loblolly extracts, both species highly susceptible to beetle kill, were at opposite extremes of fungus growth promotion, and extract from beetle-resistant slash pine promoted fungal growth.

Growth of C. minor on boiled rice was very slow. Addition of V-8 vegetable juice (200 ml. rice to 250 ml. V-8) improved it somewhat, and malt extract even more. Best growth was obtained in rice boiled in pine wood extract plus malt agar. Pine extract was made by boiling 2 l. wood shavings from a loblolly pine bolt in 2 l. water, filtering, washing to 2 l., and autoclaving. The best mixture tested was 200 ml. rice, 250 ml. wood extract, and 4.5 g. malt agar.

Inoculation experiments

In a preliminary greenhouse study 10 1-year-old seedlings each of shortleaf and slash pine were used. On October 27 a spiral strip of bark making two complete turns was removed from each seedling. Within each species five of the wounds were covered with a rice culture of C. minor, the remaining five with sterile boiled rice. The bark was replaced on each seedling and covered with masking tape to prevent drying. At 10,

12, and 14 weeks after inoculation the plants were examined and rated for vigor. The wood from dead trees was examined for blue stain.

Results of the greenhouse inoculation are summarized in table 2. Within 14 weeks the fungus had killed all five of the inoculated shortleaf seedlings and all but one of the inoculated slash pines. All of the inoculated killed trees had blue-stained wood. One control tree, a shortleaf, died, but did not show blue-stained wood.

In the field experiment 10 vigorous 6-year-old shortleaf, loblolly, and slash pine saplings were selected in a plantation. On August 30 the bark was removed from each tree in three 1½-inch wide strips, each strip extending halfway around the tree at breast height. The strips overlapped about half their length and were spaced 1½ inches apart vertically. The trees were not effectively girdled because of lateral phloem transport, but by coalescing the fungus could easily girdle the trees.

Five of the trees within each species were inoculated by smearing the wounds with a rice-wood and malt extract culture of C. minor; the five controls were similarly treated with sterile culture medium. The original bark was then replaced and the trunk sealed with masking tape and asphaltum tree paint.

After 1 year all but one, which had been windthrown, of the 30 trees were alive and apparently normal. The wounding or the rice caused some needle fall and yellowing of both inoculated and control trees for the first few months but all recovered.

Table 2.--Condition of pine seedlings 10, 12, and 14 weeks after inoculation with C. minor. Control seedlings treated with sterile rice. Scores given were: 0 = dead, 1 = poor, 2 = fair, 3 = good, 4 = excellent vigor.

Species	10 weeks		12 weeks		14 weeks	
	Inoc.	Check	Inoc.	Check	Inoc.	Check
Shortleaf	0	2	0	2	0	0
	1	3	0	3	0	3
	0	3	0	3	0	4
	3	3	3	3	0	3
	3	3	2	3	0	3
Mean score	1.4	2.8	1.0	2.8	0	2.6
Percent dead	40	0	60	0	100	20
Slash	1	4	0	3	0	4
	0	4	0	4	0	4
	4	4	3	4	0	4
	4	4	3	4	3	4
	1	4	0	4	0	4
Mean score	2.0	4.0	1.2	3.8	0.6	4.0
Percent dead	20	0	60	0	80	0

To see if the blue-stain fungus had become established in the inoculated trees, increment cores were taken 5 months after inoculation, 1 foot above the inoculated area. The cores were alcohol-flamed and aseptically cut into nine equal discs about 3 mm. thick. The discs were placed in order from the cambium inward on sterile malt agar. Growth was slow but within 2 months many plates showed Ceratocystis colonies, in addition to many contaminants. There was no horizontal gradient of infection, i.e. both Ceratocystis and other fungi seemed equally distributed from cambium to tree center. There was a highly significant effect of species on Ceratocystis infection (chi square value 28) (table 3). Shortleaf pine showed the least infection (80 percent resistant) and slash the most (20 percent resistant). These data are consistent with wood extract effects (table 1), where shortleaf extract failed to promote growth of C. minor in vitro. Again there is no positive correlation between susceptibility of shortleaf to beetle kill and susceptibility to the blue-stain fungus, or of beetle resistance in slash and resistance to blue stain. Only loblolly seems highly susceptible to both agents. One might hypothesize that here the fungus is the primary killing agent in beetle-infested trees, except that C. minor alone did not kill any loblolly saplings.

Table 3.--Number of increment core discs showing and not showing colonies of C. minor when cultured on malt agar

Inoculated tree number	Pine species					
	Shortleaf		Loblolly		Slash	
	+	-	+	-	+	-
1	0	9	9	0	8	1
2	0	9	0	9	0	9
3	8	1	9	0	8	1
4	0	9	0	9	9	0
5	0	9	4	5	8	1
Total	8	37	22	23	33	12
Percent of trees resistant	80		40		20	

DISCUSSION AND CONCLUSIONS

This study was based on the hypothesis that killing of pines infested with D. frontalis is primarily dependent on infection of the wood by C. minor, introduced by the beetle. The hypothesis was suggested by the known close association between southern pine beetle infestations and the fungus causing blue-stained wood. The fungus might kill the beetle-weakened tree by water stress from plugging or aeration of the tracheids (see Literature Review in the Study Plan). According to this hypothesis trees resistant to the fungus would automatically be susceptible to "beetle-kill." Also according to this hypothesis C. minor alone, without the beetle, should be capable of killing trees. This has indeed been demonstrated by several workers (see Study Plan). Older trees in this study were not killed by C. minor; they may not have been completely girdled by the fungus (Bramble and Holst 1940), even though they were inoculated in overlapping strips.

Three experiments were conducted to test the hypothesis. (1) C. minor was grown in vitro with wood extracts of beetle-resistant slash and longleaf and beetle-susceptible shortleaf and loblolly pines. All extracts except shortleaf promoted growth. The hypothesis was supported only with loblolly, since shortleaf should have promoted but not slash and longleaf. (2) One-year-old slash and shortleaf seedlings inoculated with C. minor were killed in 3 months, and all showed blue staining. As the hypothesis suggests, pines are capable of being killed by C. minor alone. However, these plants were too young for beetle attack, and

beetle-resistant slash was not resistant to the fungus. (3) Six-year-old saplings of slash, loblolly and shortleaf were not affected by C. minor for over a year after inoculation. Although C. minor was recovered from all three species, shortleaf showed considerable resistance. Again only in the case of loblolly was there a correlation between beetle and fungus susceptibility. However, since loblolly was not killed by the fungus alone, results of this study indicate that trees are not killed primarily by the blue-stain fungus. It may well require the combined effect of both agents. This could only be tested by infesting trees with fungus-free beetles, and possibly would require protecting the stem from air contamination.

RECOMMENDATIONS

That this study be closed, and a note be published either in Plant Disease Reporter or Phytopathology.

LITERATURE CITED

Bramble, W. C., and Holst, E. C.

1940. Fungi associated with Dendroctonus frontalis in killing shortleaf pines and their effect on conduction. Phytopathol. 30: 881-899.